MicroSnap EB (Enterobacteriaceae)

Rapid Detection of Enterobacteriaceae Bacteria
Part No: Enrichment Device, ATP-MS1-EB (100 devices) &
Detection Device, ATP-MS2-EB (100 devices)

Description/ Intended Use:

MicroSnap EB (Enterobacteriaceae) is a rapid bioluminogenic test for detection and enumeration of Enterobacteriaceae bacteria in a sample providing results in 6 to 8 hours. MicroSnap EB consists of an Enrichment Swab Device containing proprietary growth media and a Detection Device containing bioluminogenic reagents in which biomarkers produced by bacteria are measured using a small portable luminometer.

The two step test procedure requires a short incubation period facilitating growth of bacteria followed by a detection step. During incubation in enrichment media, bacteria numbers increase and potential sample interference is reduced. As bacteria grow they use up available food resources in the media and generate biomarkers. The greater the number of bacteria in the sample, the higher the biomarker concentration and the more light output. An aliquot of the enriched sample is transferred to the Detection Device, activated, mixed and measured in a luminometer. The light output is directly proportional to the concentration of bacteria present.

MicroSnap EB can be used to test environmental surfaces, product samples, water and other filterable liquids.

Required Materials (Not Provided):

- Incubator at 37 ± 0.5 °C
- EnSURE luminometer

For product samples:

- Diluents e.g.
  - Buffered Peptone Water
  - Maximum Recovery Diluent
  - Butterfields
  - Other validated diluents of user's choice
- Sample bags
- Homogenizing equipment

Table 1: Dynamic Range (Limit of Detection)

Sample Type	CFU Range
Surface	0 – 5,000
1mL Liquid	0 – 5,000
10% w/v Suspension of Solid	0 – 50,000 (bacteria/g)*

*For samples where contamination is outside ranges detailed in Table 1, then the following serial dilutions must be made in order to be read on the luminometer:

- 1% suspension will be 1,000 – 500,000 CFU
- 0.1% suspension will be 10,000 – 5,000,000 CFU

Directions:

Instructional Video: ***http://www.scigiene.com/Microsnap***

Step 1: Enrichment

Enrichment procedure is described below and is also shown in Step 1 diagrams.

1. Collect sample and place in the MicroSnap EB Enrichment Device (Part # MS1-EB). Samples can be:
   i. Surface - Swab a 4 x 4 inch (10 x 10 cm) square area, or for irregular surfaces, as much of surface as possible to collect a representative sample.
   ii. Liquid - 1mL liquid food, beverage or water samples added directly to Enrichment Device.
   iii. Product – 1mL of appropriate suspension, e.g. 10% w/v (weight/volume) food homogenate added directly to Enrichment Device. Food homogenate should be prepared using standard microbiological procedures. For unknown sample contamination, dilutions below 10% should be made and tested.
2. Re-attach swab back on to swab tube. Device should look the same as it did when first pulled from bag.
3. Activate Enrichment Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward.
4. Separate bulb and swab tube about 1-2 inches from each other, relieving internal pressure, and squeeze bulb to flush all media to bottom of swab tube. Ensure most of enrichment broth is in bottom of swab tube.
5. Re-attach swab back on to swab tube firmly to seal device.
6. Shake tube gently to mix sample with enrichment broth.
7. Incubate at 37 ± 0.5 °C for 6 to 8 hours. (Refer to Tables 2 – 4 for details).

Step 2: Detection

Detection procedure is described below and is also shown in Step 2 diagrams. Before beginning Step 2, turn on EnSURE luminometer. If locations have been programmed, select location to be tested.

1. Allow the MicroSnap EB Detection Device (Part # MS2-EB) to equilibrate to room temperature (10 minutes at 22 – 26 °C). Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once. This will bring extractant liquid to bottom of tube.
2. Transfer enriched sample from Enrichment Device to Detection Device. Enrichment Swab can be used as a pipette for convenience.
   i. Squeeze and release Enrichment Device bulb to mix and draw sample into bulb.
   ii. Remove Enrichment swab from tube.
   iii. Open Detection Device by twisting and pulling to remove bulb. Set aside.
      a. Insert Enrichment swab tip into top of Detection Device tube (approximately 1 inch or 3 cm) and lightly squeeze Enrichment Device bulb to trickle enriched sample into tube until volume reaches fill line marked on bottom of Detection Device tube. Avoid adding excess sample above fill line, as this can increase variation of test results.
   iv. Reassemble Detection Device to original state.
3. Activate Detection Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward. Squeeze bulb 3 times to release all liquid to bottom of swab tube.
4. Shake gently to mix.
5. Immediately insert whole device into luminometer; close lid and holding unit upright, press "OK" button to initiate measurement. Results will appear after 15 second count down.
6. Result will be displayed in RLU (Relative Light Units). Set RLU thresholds on instrument to correspond with required CFU limits. Refer to "Interpretation of Results" below for correlation.

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& Detection Device, ATP-MS2-EB (100 devices)

Interpretation of Results:

Results are displayed as Relative Light Units (RLU). RLU output is proportional to the starting inoculums and corresponding bacteria equivalent numbers (expressed as Colony Forming Units, CFU). Tables 2 – 4 show the equivalent CFU values for RLU measurements at various incubation times.

Table 2: Correlation between RLU and CFU at 6 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	<50/mL	<500/g
<25	<120/mL	<1,000/g
<50	<250/mL	<2,500/g
<100	<500/mL	<5,000/g
<250	<1,200/mL	<12,000/g
<500	<2,500/mL	<25,000/g
<1,000	<5,000/mL	<50,000/g
>1,000	TNTC	TNTC

Table 3: Correlation between RLU and CFU at 7 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	<5/mL	<50/g
<25	<12/mL	<100/g
<50	<25/mL	<250/g
<100	<50/mL	<500/g
<250	<120/mL	<1,200/g
<500	<250/mL	<2,500/g
<1,000	<500/mL	<5,000/g
>1,000	TNTC	TNTC

Table 4: Correlation between RLU and CFU at 8 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	Absence	Absence
<25	Absence	Absence
<50	Absence	<25/g
<100	<5/mL	<50/g
<250	<12/mL	<120/g
<500	<25/mL	<250/g
<1,000	<50/mL	<500/g
>1,000	TNTC	TNTC

Where several dilutions are prepared and tested for samples with unknown contamination, the CFU/ g or mL is calculated by multiplying the RLU result by the corresponding dilution factor. A convenient Microsoft Excel® calculator is available for calculating RLU to CFU conversions. Contact a Scigiene representative for details.

Calibration Control:

It is advisable to run positive and negative controls according to Good Laboratory Practices. Scigiene offers the following control:

- Calibration Control Kit (Part # ATP4000)

Storage & Shelf Life:

- Store at 2 – 8 °C.
- Devices have a 12 month shelf life.
- Check expiration date on label.

Disposal:

Disinfect before disposal. MicroSnap devices can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour. Then, they can be placed in the trash. Alternatively, MicroSnap devices may be discarded at a biohazard waste disposal facility.

Safety & Precautions:

Components of MicroSnap devices do not pose any health risk when used correctly. Used devices confirming positive results may be a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations. Do not use devices after expiration date.

1. MS-EB Detection Device is designed for a single use. Do not reuse.
2. Do not use devices after expiration date.
3. Sampling should be done aseptically to avoid cross contamination.
4. Ensure proper dilution of sample to be read within the luminometer's dynamic range (see Table 1).
5. Ensure proper incubation temperature and time for the test application.

Hygiena Liability:

As with any culture medium, MicroSnap EB results do not constitute a guarantee of quality of food, beverage products or processes that are tested with these Devices. Hygiena will not be liable to user or others for any loss or damage, whether direct or indirect, incidental or consequential from use of this Device. If this product is proven to be defective, Hygiena's sole obligation will be to replace product, or at its discretion, refund the purchase price. Promptly notify Scigiene within 5 days of discovery of any suspected defect and return product to us. Please call Customer Service for a Returned Goods Authorization Number.

Contact Information:

If more information is required, please visit us at www.scigiene.com or contact us at:

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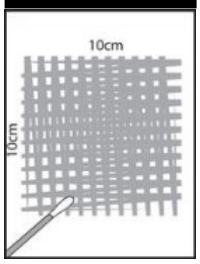
MicroSnap™ EB (Enterobacteriaceae)

Step: 1 Enrichment of Environmental Surface Swab, Liquid and Solid Samples

1

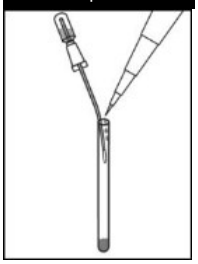
Surface



1-i. **Surface:** Swab a 10x10cm area with Enrichment Device.

OR

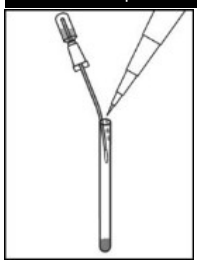
Liquids



1-ii. **Liquids:** Add 1mL liquid food, beverage or water sample directly to Enrichment Device.

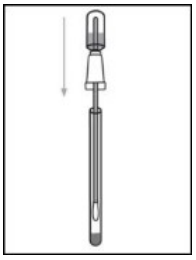
OR

Solid Samples



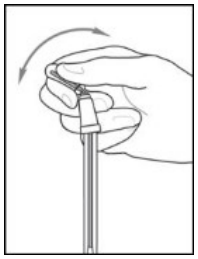
1-iii. **Solid Samples:** Add 1mL of appropriate dilution of solid samples directly to Enrichment Device.

2



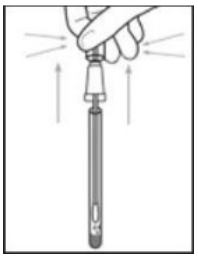
2. Re-insert Snap-Valve bulb into swab tube.

3



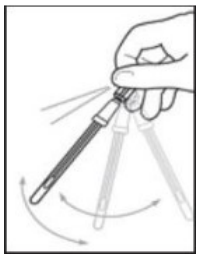
3. Activate Device. Bend bulb, breaking Snap-Valve.

4



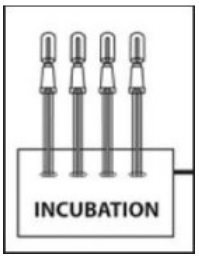
4. Lift bulb up (about 1 – 2 inches) and squeeze bulb to release liquid into bottom of tube. Replace bulb on to tube. Liquid should now be in bottom of tube.

5



5. Shake tube gently to mix sample in liquid.

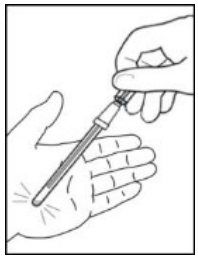
6



6. Incubate at 37 ± 0.5 °C for 6 - 8 hours. Proceed to Step 2.

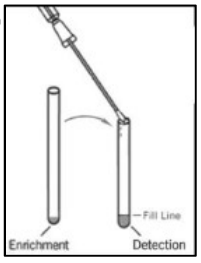
Step: 2 Detection / Measurement

1



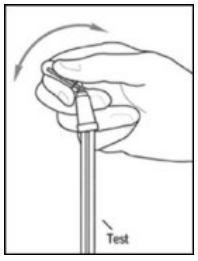
1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.

2



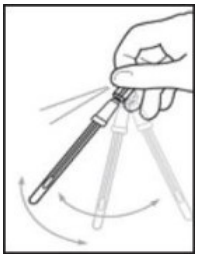
2. Transfer enriched sample from Enrichment Device to Detection Device.

3



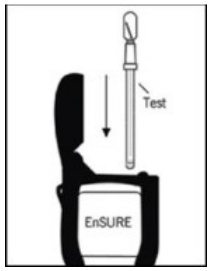
3. Activate Detection Device by breaking Snap-Valve. Squeeze bulb to release liquid into tube. Liquid should now be in bottom of tube.

4



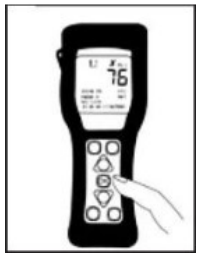
4. Shake tube gently to mix sample in liquid.

5



5. Insert Detection Device into a luminometer and initiate measurement.

6



6. Record results as RLUs. Refer to Table 2 for interpretation of results.

Instructional Video:
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